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Fluorescence Determination of DNA Using the Gatifloxacin−Europium(III) Complex

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Supporting Information

ABSTRACT: A method for the determination of DNA based on the fluorescence intensity of the gatifloxacin−europium(III) (GFLX−Eu^{3+}) complex that could be enhanced by DNA was developed. The GFLX−Eu^{3+} complex showed an up to 6-fold enhancement of luminescence intensity after adding DNA. Under the optimized experimental conditions, the system exhibited a linear relationship between the enhanced fluorescence intensity and the concentration of calf thymus DNA (ctDNA) over the range from 1.0×10^{-8} to 1.5×10^{-6} g mL^{-1}, with a correlation coefficient (R) of 0.997, and the detection limit (3σ) of the method was determined as 6.0×10^{-9} g mL^{-1}. The mechanism of the fluorescence enhancement effect was also discussed.

KEYWORDS: DNA, gatifloxacin−europium, determination, fluorescence

INTRODUCTION

The quantitative determination of micro-amounts of nucleic acid has attracted a great deal of attention in the fields of medicine and molecular biology. Many methods have been developed, such as direct determination, including ultraviolet absorption and determination of ribose or deoxyribose in nucleic acid,[1] spectrophotometry,[2] chemiluminescence,[3] electrochemical chromatography,[4] including high-performance liquid chromatography[5] and paper chromatography,[6] capillary electrophoresis,[7] and resonance light scattering.[8] However, low sensitivity and easy interruption by protein and other biomolecules existed in these methods in common. However, the fluorometric methods make predominant concern because of their high sensitivity and selectivity. Generally, the fluorescence intensity of DNA must be enhanced by fluorescent probes because that it emits weak fluorescence itself.[9] Fluorescent probes, such as ethidium bromide (EB) and its derivatives,[10] Hoechst 33258,[11] YOYO and TOTO dyes,[12] and SYBR Gold and SYBR Green I,[13,14] are preferred tools. However, many of these fluorescent DNA probes are toxic to the environment and human body. Therefore, the search for a friendly to the environment, selective, and sensitive DNA probe is a new field of research.

In recent years, metal−ligand complexes[15,16] and the rare earth (Sm, Gd, La, Nd, Eu, and Tb) coordination compounds[17−20] are obtaining more attention as a novel probe to study DNA. In particular, the rare earth coordination compounds owe narrower emission spectra and other predominant fluorescence characteristics than metal−ligand complexes. The fluorescence of rare earth coordination compounds is often the result of efficient intermolecular energy transfer from the excited triplet state of the antenna ligand to the emitting electronic level of the rare earth ion. The formed complexes show a narrow spectral width, long fluorescence lifetime, large stock shift, and strong binding with biological molecules. Therefore, they are frequently employed to investigate the function of nucleic acids using their ability to intercalate into the double strand. Especially, Tb^{3+} and Eu^{3+}, because their resonance energy levels overlap with ultraviolet light, could be used to determine DNA quantitatively in the ultraviolet region.[21−24] Gatifloxacin (GFLX) belongs to the fourth-generation quinolones, which is widely used as an antibacterial agent that inhibits DNA gyrase and topoismerase IV by binding to double-stranded DNA (dsDNA).[25] The structure of GFLX is shown in Figure 1.

GFLX could form the coordination complex with Eu^{3+}, which emitted the characteristic fluorescence of Eu^{3+}.[26] However, the fluorescence intensity of the system increased 6-fold when DNA was added. On the basis of the above findings, the fluorescence enhancement effect of the GFLX−Eu^{3+} complex by DNA was investigated in the paper in detail. Under the optimal conditions, the enhancement of the fluorescence intensity could be used for the quantification of DNA over a linear range from 1.0×10^{-8} to 1.5×10^{-6} g mL^{-1}. In comparison to previous DNA probes, the GFLX−Eu^{3+} complex is not only more stable and soluble in neutral pH solution but friendly to the environment. Meanwhile, it is more sensitive than most metal−ligand complexes and other Eu^{3+}−ligand complexes.

MATERIALS AND METHODS

Apparatus. The fluorescence spectra and intensities were measured with a Hitachi F-2500 fluorescence spectrophotometer (Japan), using a standard 10 mm path-length quartz cell with 10 nm bandwidths for both the excitation and emission monochromators. All absorption spectra were measured on a UV-2550PC spectrophotometer (Shimadzu, Japan) equipped with 10 mm path-length quartz cells. The pH was measured using a Lei Ci pHs-3C pH-meter (Shanghai, China). A Vario

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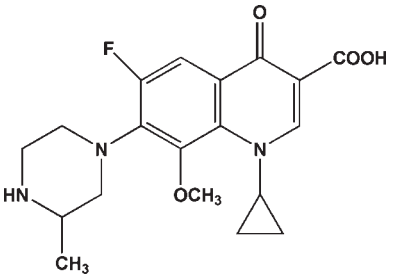

Figure 1. Chemical structure of GFLX.

EI III elemental analyzer (Germany) was employed for the analysis of elements C, H, and N in the GFLX−Eu^{3+} complex. An IRIS Intrepid II XSP inductively coupled plasma−atomic emission spectrometry (ICP−AES) (Thermo Scientific Corp., Waltham, MA) was applied to the analysis of Eu^{3+} in the GFLX−Eu^{3+} complex. The infrared spectra (IR) were recorded on Thermo Nicolet Nexus 470 ESP Fourier transform infrared (FTIR) spectroscopy (Thermo Scientific Corp., Waltham, MA) The rotary evaporation was performed on a Shen Sheng rotary evaporator (Shanghai, China).

Reagents. The standard stock solutions (1.0×10^{-4} g mL^{-1}) of DNA were prepared by dissolving commercially prepared calf thymus DNA (ctDNA), herring sperm DNA (hsDNA), and salmon sperm DNA (salsDNA) (from Sigma) in water, respectively. Standard working solutions were obtained by making appropriate dilutions of the standard stock solution with water. A standard stock solution (1.0×10^{-4} mol L^{-1}) of the GFLX−Eu^{3+} complex was prepared by dissolving the synthesized GFLX−Eu^{3+} complex in water and then diluting to the corresponding volume. A 1.0 mol L^{-1} hexamethylenamine (HMA)−HCl buffer solution was prepared by dissolving corresponding HMA in water and adjusting the pH to 6.5 with HCl and then giving a final total volume with water. All of the stock solutions and their diluted solutions were stored in a refrigerator at 4 °C and protected from light until used. All diluted solutions were used within 24 h. All chemicals were of analytical reagent grade, and the deionized water was used throughout the study.

Synthesis of the GFLX−Eu^{3+} Complex. Proper Eu_2O_3 was weighed, dropped by an appropriate amount of HCl, and then heated carefully to make it dissolve. Afterward, the solution was kept heating until a large amount of crystal precipitated after cooling. Thus, the hydrated lanthanide chloride $EuCl_3 \cdot 6H_2O$ was obtained. $EuCl_3 \cdot 6H_2O$ and GFLX were mixed together according to the molar ratio 1:3.1 in water. Then, the solution was electromagnetically stirred for 1 h at room temperature. Then, a yellow solution was acquired after vacuum filtration. At last, the solution was rotary-evaporated until the solvent water was absolutely removed. Then, the product was milled into powder and stored in a silica gel desiccator.

Determination of Fluorescence Intensity. To a 10.0 mL volumetric flask, three solutions were added according to the following order: 0.5 mL of GFLX−Eu^{3+} complex working solution (1.0×10^{-4} mol L^{-1}), 0.5 mL of HMA−HCl buffer (1.0 mol L^{-1}, pH 6.5), and 0.5 mL of DNA working solutions (1.0×10^{-4} g mL^{-1}). Then, the mixture were diluted to 10 mL with water, mixed thoroughly by shaking, and allowed to stand for 10 min at room temperature. The fluorescence intensity was measured in a 10 mm path-length quartz cell, with the excitation and emission wavelengths of 331 and 617 nm, respectively. The enhanced fluorescence intensity of the GFLX−Eu^{3+} complex by DNA was represented as ΔI.

RESULTS AND DISCUSSION

Spectral Characteristics of GFLX−Eu^{3+}−DNA. The excitation peak of the GFLX−Eu^{3+} system was 331 nm, which was selected as the excitation wavelength. In the emission spectrum, a weak characteristic fluorescence peak of GFLX−Eu^{3+} can be observed in 617 nm; however, it was enhanced 6-fold when DNA was added (Figure 2). The strong emission peak at 617 nm corresponded to the transition of the Eu^{3+} $^5D_0$−$^7F_2$.[27] It indicated that the interaction between the GFLX−Eu^{3+} complex and DNA was generated in the GFLX−Eu^{3+}−DNA system. Therefore, a wavelength of 617 nm was selected as the emission wavelength for the determination of dsDNA.

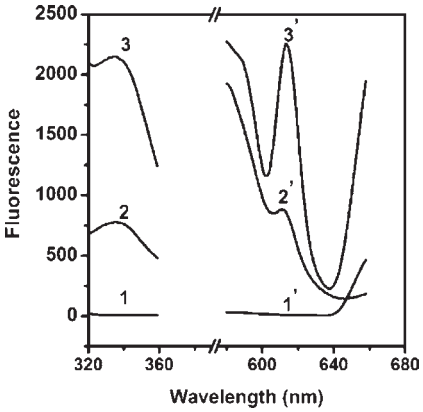

Figure 2. Excitation and emission spectra: (1 and 1′) ctDNA, (2 and 2′) GFLX−Eu^{3+} complex, and (3 and 3′) GFLX−Eu^{3+}−DNA system (C_{ctDNA}, 5.0×10^{-6} g mL^{-1}; $C_{GFLX-Eu^{3+}}$, 5.0×10^{-6} mol L^{-1}; pH, 6.5).

The changes of UV spectra of the GFLX−Eu^{3+} system in the absence and presence of ctDNA were shown in Figure 3. Under the condition of pH 6.5, there were two absorption peaks at 286 and 331 nm, respectively. With the addition of ctDNA, the absorbance of two peaks all decreased and the 286 nm absorption peak shifted to 288 nm. This can be explained by the fact that there was little hypochromism and a weak red shift of 2 nm, which were caused by the shielding effect of the base pairs of DNA and the perturbation of the complex chromophore system upon binding to ctDNA, respectively.[24] These results also correlated well with the fluorescence enhancement of the GFLX−Eu^{3+} system after adding ctDNA. They all implied the intercalation of the GFLX−Eu^{3+} complex into the duplex structure of DNA.

Influence Factors on the Fluorescence Intensity of the System. *Effect of pH and the Concentration of the GFLX−Eu^{3+} Complex and DNA.* GFLX is an amphoteric species with a piperazinyl and a quinolone ring (Figure 1); therefore, the fluorescence intensity of the GFLX−Eu^{3+} system with DNA is strongly dependent upon the pH value (Figure 4). The result indicated that the maximum fluorescence intensity of the system stabilized in the pH range of 6.3−6.7. Therefore, we selected pH 6.5 for further experiments. The effect of the buffer solutions, HMA−HCl, Tris−HCl, NaAc−HAc, Na_2HPO_4, sodium citrate, and hydrogen potassium phthalate, on the fluorescence intensity was then examined. It revealed that 5.0×10^{-2} mol L^{-1} HMA−HCl buffer offered the highest sensitivity. The effect of the GFLX−Eu^{3+} complex and the ctDNA concentration on the fluorescence intensity of the GFLX−Eu^{3+}−DNA system was also investigated, respectively. At last, the optimal conditions were selected as follows: the GFLX−Eu^{3+} complex was 5.0×10^{-6} mol L^{-1}, HMA−HCl was 5.0×10^{-2} mol L^{-1}, and ctDNA was 5.0×10^{-6} g mL^{-1}.

Fluorescence Stability. The experiments showed that, at room temperature, the fluorescence intensity of the GFLX−Eu^{3+}−DNA

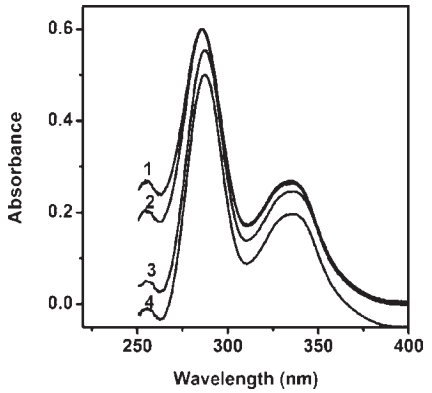

Figure 3. Absorption spectra of the GFLX−Eu^{3+} system in different concentrations of ctDNA: (1) 0 g mL^{-1}, (2) 2.5 × 10^{-5} g mL^{-1}, (3) 5.0 × 10^{-5} g mL^{-1}, and (4) 1.0 × 10^{-4} g mL^{-1} ($C_{GFLX-Eu^{3+}}$, 5.0 × 10^{-6} mol L^{-1}; pH, 6.5). Spectra were referenced against ctDNA solutions of exactly the same ctDNA concentration and were adjusted to a common baseline.

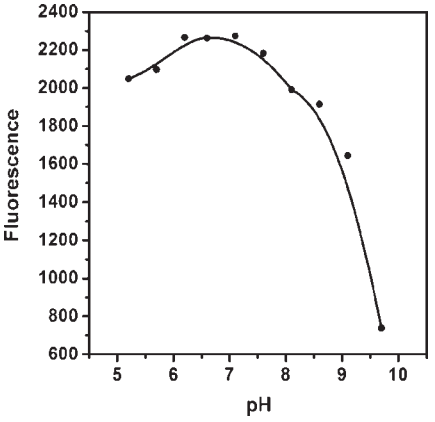

Figure 4. Effect of pH to the GFLX−Eu^{3+}−DNA system (C_{ctDNA}, 5.0 × 10^{-6} g mL^{-1}; $C_{GFLX-Eu^{3+}}$, 5.0 × 10^{-6} mol L^{-1}).

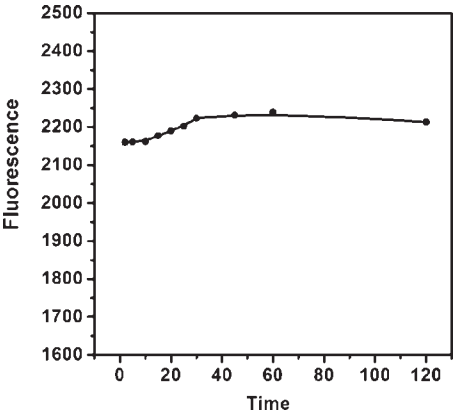

Figure 5. Stability time of the GFLX−Eu^{3+} system interaction with DNA (C_{ctDNA}, 5.0 × 10^{-6} g mL^{-1}; $C_{GFLX-Eu^{3+}}$, 5.0 × 10^{-6} mol L^{-1}; pH, 6.5).

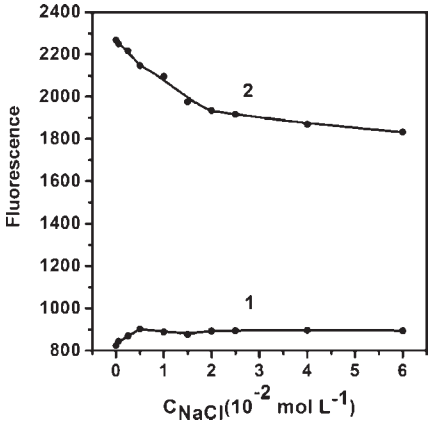

Figure 6. Effect of salt concentrations on the fluorescence intensity of the GFLX−Eu^{3+} system (1) in the absence of ctDNA and (2) in the presence of ctDNA (C_{ctDNA}, 5.0 × 10^{-6} g mL^{-1}; $C_{GFLX-Eu^{3+}}$, 5.0 × 10^{-6} mol L^{-1}; pH, 6.5).

system reached its maximum in 10 min after all of the reagents had been added and remained stable for at least 2 h (Figure 5).

Effect of Ionic Strength and Interferences. The ionic strength is known to give a large effect on most DNA probes. Sodium chloride was employed to investigate the influence of the salt concentration on the fluorescence intensity of the GFLX−Eu^{3+}−DNA system. The fluorescence intensity of the GFLX−Eu^{3+} system in the absence of ctDNA suffered a slight decrease with the increasing ionic strength; however, the change in the presence of ctDNA was significant. A 20% decrease in the fluorescence intensity was observed with sodium chloride solution in the concentration range from 1.0 × 10^{-3} mol L^{-1} to 6.0 × 10^{-2} mol L^{-1} (Figure 6). Hence, it was assumed that the interaction of the GFLX−Eu^{3+} system with ctDNA was electrostatic. To assess the applicability of the proposed method in biological samples, the effect of the potential interferents, such as metal ions, amino acids, proteins, sugars, pyrimidines, purines, and surfactants, was examined. The tolerance levels of various interferents were summarized in Table 1. It was obvious that most species had little effect on the fluorescence intensity, except for dihydrophosphate, pyrimidine, and purine. They generated a significant effect if their concentrations exceeded 5.0 × 10^{-6} mol L^{-1}. These three substances all affected the interaction of the GFLX−Eu^{3+} complex and DNA.

Determination of DNA. *Calibration Curve and Detection Limit.* Under optimal conditions (the optimum conditions of hsDNA and salsDNA were the same as the ctDNA), the enhanced fluorescence intensity of the system (ΔI) showed an excellent linear relationship with the concentration of ctDNA, hsDNA, and salsDNA (Table 2). The linear equation was $\Delta I = 42.49 + 0.2151C_{ctDNA}$ (ng mL^{-1}), with a correlation coefficient of 0.997. The detection limit (3σ) was 6.0 × 10^{-9} g mL^{-1}. In comparison to the metal coordination complex and some Eu^{3+} coordination complexes referred in Table 3, the method proposed here offered even more sensitivity.

Determination of DNA in Synthetic Samples. To validate the specificity of this method, a sample containing bovine serum albumin (BSA), glucose, an amino acid (L-alanine), and several metal ions (Na^+, K^+, and Ca^{2+}) was tested by the standard addition method. The method worked satisfactorily in both the recovery and precision for the three types of DNA. The result (Table 4) proved that all recoveries were in the range of 96.9−104.0%. Therefore, this simple method could represent an efficient tool for studying DNA.

Mechanisms of the Fluorescence Enhancement. From the chemical structure of the GFLX−Eu^{3+} complex (Figure 7), after characterization (see the Supporting Information), we could obtain that every Eu^{3+} could coordinate with three GFLX molecules and two H_2O molecules. Because the GFLX−Eu^{3+} complex was synthesized in the neutral pH solution, when Eu^{3+} coordinated with GFLX, H^+ of the carboxyl group would transfer to end-group N and form the $-H_2N^+$ group; correspondingly, Cl^- would balance the charge of the whole complex. This special charge separation structure will not only increase the solubility of the GFLX−Eu^{3+} complex greatly but adjust the interaction of the GFLX−Eu^{3+} complex and DNA.

There was no fluorescence signal of the GFLX aqueous solution and the Eu^{3+} aqueous solution at 617 nm, but in the GFLX−Eu^{3+} system, there was weak emission fluorescence at 617 nm,[30] which was caused by the energy transfer from GFLX to Eu^{3+}. As shown in Figure 2, the fluorescence intensity of the GFLX−Eu^{3+} system was improved after the addition of DNA. This phenomenon indicated that the energy transfer of the GFLX−Eu^{3+} system was incomplete, and the addition of DNA could enhance that. It could be explained as follows: The coordination number of Eu^{3+} is eight, from the coordination structure of GFLX−Eu^{3+}, which indicates that the eight coordination site of every Eu^{3+} could be saturated by six O of three GFLX molecules and two O of two H_2O molecules in the solvent. As a result, the O−H vibration will adsorb the excited state energy of Eu^{3+} and make a transition to the fundamental frequency

Table 1. Effects of Coexistence Interferents (C_{ctDNA}, 5.0 × 10^{-6} g mL^{-1}; $C_{GFLX-Eu^{3+}}$, 5.0 × 10^{-6} mol L^{-1}; pH, 6.5)

substance	concentration (mol L^{-1})	change of luminescence intensity (%)
BSA	1.0 × 10^{-6}	−7.1
$H_2PO_4^-$	1.0 × 10^{-6}	−8.0
thymine	4.0 × 10^{-6}	−5.7
adenine	5.0 × 10^{-6}	−8.4
Ca^{2+}	1.0 × 10^{-4}	−8.5
tryptophane	3.3 × 10^{-4}	−3.8
Mg^{2+}	5.0 × 10^{-4}	−9.0
ascorbic acid	1.0 × 10^{-3}	−8.2
L-alanine	2.0 × 10^{-3}	−8.1
L-glutamic acid	2.0 × 10^{-3}	−8.4
Na^+	5.0 × 10^{-3}	−7.8
K^+	5.0 × 10^{-3}	−7.9
glucose	5.0 × 10^{-3}	−9.3
L-glycine	5.0 × 10^{-3}	−9.7
lactose	5.0 × 10^{-3}	−10.0

Table 4. Results of the Determination of DNA in Synthetic Samples ($C_{GFLX-Eu^{3+}}$, 5.0 × 10^{-6} mol L^{-1}; pH, 6.5; *n*, 5; C_{HSA}, 1.0 × 10^{-6} g mL^{-1}; C_{Na^+}, C_{K^+}, and $C_{Ca^{2+}}$, 1.0 × 10^{-5} mol L^{-1}; $C_{glucose}$, 1.0 × 10^{-5} mol L^{-1}; $C_{L\text{-alanine}}$, 5.0 × 10^{-6} mol L^{-1})

nucleic acid	added (ng mL^{-1})	found (ng mL^{-1})	recovery (%)	RSD (%)[a]
ctDNA	500	499.3	99.8	5.3
	1000	1034.2	103.4	3.8
hsDNA	500	502.8	100.6	3.2
	1000	969.3	96.9	3.6
saleDNA	500	505.0	101.0	4.0
	1000	1039.9	104.0	4.2

[a] RSD (%) = relative standard deviation.

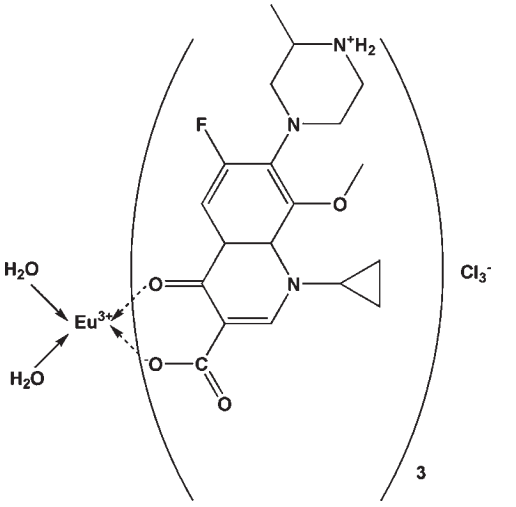

Figure 7. Chemical structure of the GFLX−Eu^{3+} complex.

Table 2. Analytical Parameters for DNA Determination ($C_{GFLX-Eu^{3+}}$, 5.0 × 10^{-6} mol L^{-1}; pH, 6.5)

DNA	linear range (ng mL^{-1})	linear regression equation (ng mL^{-1})	correlation coefficient	detection limit (ng mL^{-1})
ctDNA	10−1500	$I - I_0 = 42.49 + 0.2151C$	0.997	6.0
hsDNA	10−1500	$I - I_0 = 25.05 + 0.3630C$	0.998	6.0
salsDNA	10−1500	$I - I_0 = 25.72 + 0.1360C$	0.994	6.0

Table 3. Overview on Fluorescence Probes for the Determination of DNA

fluorescence probe	nucleic acid	LOD (×10^{-9} g mL^{-1})	reference
Al^{3+}−salicylidene−*o*-aminophenol	ctDNA	49	15
Zn−pyrazine−2,5-di-[2-(3,5-bis(2-pyridylmethyl)amine-4-hydroxyphenyl)ethylene]	ctDNA	19	16
Eu^{3+}−oxytetracycline	ctDNA	15.1	28
Eu^{3+}−tetracycline	hsDNA	10	29
Eu^{3+}−GFLX	ctDNA	6.0	this probe

vibration area. Consequently, the vibrational relaxation will weaken the fluorescence intensity of the GFLX−Eu^{3+} complex seriously. When DNA was added, the electron-donor atom (O and N) of the basic group in DNA could coordinate with Eu^{3+} and the phosphate group of DNA could interact with Eu^{3+} by a hydrogen bond, then improve the coordinate microenvironment, and decrease the binding of the H_2O molecule and Eu^{3+}. Thus, the radiationless energy loss through O−H vibration of the H_2O molecule in the GFLX−Eu^{3+} complex will decrease, and the fluorescence intensity will be enhanced by DNA.

■ ASSOCIATED CONTENT

Supporting Information. Characterization data of the GFLX−Eu^{3+} complex, including elemental analyses, ICP−AES, fluorescence spectra, and IR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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